We conclude that transduction channels provide a major contribution to hair-bundle friction. Channel properties, but not endolymph viscosity, control damping of hair-bundle movements. Channel friction in turn helps setting the sensitivity and the characteristic frequency of the hair-bundle amplifier.

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Higher-Order Mode-Locking and Phase Slips in Hair Cell of the Inner Ear
Yuttana Roongthumskul, Dolores Bozovic.
University of California, Los Angeles, Los Angeles, CA, USA.
Auditory system is known for its exquisite sensitivity, displaying sub-nanometer detection thresholds. Mechanical deflections evoked by external sound and ground vibrations are converted by the inner ear hair cells into electrical signals. In some species, hair cell bundles exhibit spontaneous oscillations under *in vitro* conditions. In Bullfrog sacculus, the oscillation profile is consistent with relaxation-type oscillation, in which a rapid motion of a bundle is followed by a slow drift along the same direction. A large number of hair bundles also show more complex temporal profiles, with quiescent intervals interspersed with bursts of oscillation. We study the dynamics of phase-locking of individual hair bundles to low-amplitude mechanical stimulations, where the amplitude of the oscillations remains unaffected by the stimulus. Under low-frequency stimulation, the signal entrains the bundle motility via higher-order mode-locking. The applied signal modulated both the quiescent intervals and the oscillatory bursts, across a broad range of frequencies. We compare these experimental findings with results from numerical simulations.

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Self Tuning of Hair Cell Bundle after Prolonged Deflection
Albert Kao, Sebastiaan W.F. Meenderink, Dolores Bozovic.
UCLA, Department of Physics and Astronomy, los angeles, CA, USA.
Active hair bundle motility is one of the signatures of the amplification mechanism in non-mammalian hair cells. Here we impose high-amplitude deflections on the hair bundle for 0.1-50 seconds. Due to incompleteness of the adaptation mechanism, this is expected to change the opening probability of the transduction channels, thus mechanically bringing the bundle out of its equilibrium state. Recovery of hair bundle motion was characterized, and the effects of calcium on the feedback mechanism were investigated. Traces of hair bundle motility recorded immediately post stimulation show a slow drift towards the equilibrium position, without oscillatory motion. Bundles recover their innate oscillation between 0.1 - 1 second after cessation of the stimuli, and the quiescent time (Tq) shows a strong dependence on stimulus duration. Relaxation of hair bundles is fitted by the sum of two exponentials. The shorter of the time constants is found to be in the range ~20-50 ms, consistent with myosin-motor dynamics. The longer time constant is significantly slower, ~0.5-1 s. Decreased calcium concentration leads to a slower spontaneous oscillation, consistent with prior results in the field. The quiescent interval induced by deflection of hair bundles in a low-calcium environment is shorter, recovering more rapidly post stimulus cessation. Blockage of the extrusion pumps that regulate its concentration inside the stereocilia was likewise seen to prolong the induced quiescence, indicating that calcium accumulation may underlie the suppression of spontaneous motility.

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FLIM-FRET Measurements of Voltage Dependent Conformational Changes in the Outer Hair Cell Motor Protein Prestin
Shinji Strain, Guillame Duret, Chance Mooney, Robert M. Raphael.
Rice University, Houston, TX, USA.
Cochlear sensory cells, outer hairs cells (OHCs), elongate and contract in response to changes in transmembrane potential. This electromotility is important for auditory frequency selectivity and sensitivity and is believed to be driven by a motor protein, prestin, which is highly expressed within the membrane of OHCs. Experiments have shown that prestin undergoes self-association leading to the observation that oligomerization might be important for proper function. We have previously utilized acceptor photobleach fluorescence resonance energy transfer (FRET) to study pretin-prestin interactions in living cells. However, traditional FRET, based upon detection of fluorescence intensity, is inherently subjected to artifacts that arise from variations in excitation intensity, photobleaching, and fluorophore concentration. We have recently implemented fluorescence lifetime imaging microscopy (FLIM) to perform robust FLIM-FRET measurements in HEK cells cotransfected with prestin-TFP and prestin-YFP. FRET efficiency was calculated by fitting the lifetime decay of prestin-TFP in cells clamped at various holding potentials. High FRET efficiencies were measured at hyperpolarized potentials, and low FRET efficiencies were measured at depolarized. The results indicated that changes in the transmembrane potential induce a conformational change in prestin that can be detected by FLIM-FRET. Thus, FLIM-FRET can provide a sensitive assay for the mechanical correlate of prestin function.

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Effect of Non-Steroidal Anti-Inflamatory Drugs on the Outer-Hair Cell Protein Prestin
Guillaume Duret, Robert Raphael.
Rice University, Houston, TX, USA.
Physiologically, high doses of salicylate can cause reversible hearing loss and tinnitus, potentially because of the impact on prestin function. Prestin is a member of the SLC26 family which regroups anion antiporters, and is essential to the electromotility of the outer-hair cells. Other NSAIDs can trigger side effects related with hearing and cause tinnitus. Although ototoxicity can be due to the interaction of these medicines with any sensitive part of the hearing system, we are investigating a possible prestin-related mechanism for these adverse reactions.
The coupling between electromotility and the non linear capacitance of prestin (NLC) has established the NLC as a surrogate measure of prestin function. We assessed the effect of ibuprofen, acetaminophen and naproxen, as well as the salicylate-derivatives diflusinal and piroxicam on prestin functions. All NSAIDs tested, except piroxicam, triggered a voltage-shift of the NLC. Moreover, ibuprofen and diflusinal had a significant impact on prestin's charge density. The presence of 2 mM diflusinal decreased the charge density by over 50% which could suggest a competition with Cl- ions. The perfusion of 6mM ibuprofen, on the other hand, increased the charge density by 20%, suggesting a decrease in membrane lateral pressure.
Thus, NSAIDs induce alterations in the function of prestin which are compatible with hearing alteration. The NSAID-induced shift in the NLC can modify the capacitance of the OHC at resting potential, which in turn can impact hearing. We also witnessed a correlation between the V1/2-shift and the pKas of the NSAIDs which suggests that the charges brought to the membrane by the NSAIDs are responsible for the alteration in V1/2.
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Measurement of the Organ of Corti Mechanical Responses using a New Microchamber
Jong-Hoon Nam, Talat Jabeen, Alexander P. Ringo, Daniel Marnell.
University of Rochester, Rochester, NY, USA.
The mammalian cochlea is an acoustic spectrum analyzer and pressure transducer with a remarkable operating range. In the cochlear sensory epithelium called the organ of Corti (OC), cellular mechanical force is transmitted to select and amplify acoustic vibrations. However, the amplification mechanism is still unclear. Diverse experiments in cochlear research have been developed to clarify the mechanics and physiology of the OC, but most experiments share some concerns (e.g., non-physiological electrical and chemical conditions of in vitro experiments; limited means of control over the subject of in vivo tests). Furthermore, most available data of cochlear mechanics are from the basal (high frequency) region.
We developed a new microchamber system to overcome the limitations. The microchamber has two ports for solution circulation, two ports for sound delivery and exit, two slits for magnetic pole tips, a port for a ground electrode, and a slit for the cochlear tissue placement. An 800 μm long sensory epithelium was harvested from young (7-11 days old postnatal) gerbil cochlea. The tissue was transplanted to the microchamber. The tissue separated two fluid spaces filled and refreshed with different ionic solutions imitating the original chemical conditions of the endolymph and the perilymph. A target bead was attached to the tectorial membrane (an acellular matrix on top of the OC). Acoustic pressure was delivered through the bottom chamber and the motion of the target bead was measured using a laser interferometer (along the optical axis) and photodiodes (in transverse plane). The motion trajectory was analyzed with our computational model of the OC.
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Molecular Dynamics II

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Analysis of Free Energy of Opening the Troponin C Binding Pocket for Troponin I using Microsecond Molecular Dynamics Simulations
Steffen Lindert, Pete Kekenes-Huskey, J. Andrew McCammon.
UCSD, La Jolla, CA, USA.
Troponin (Tn), part of the thin filament in cardiomyocytes, plays an important role in calcium signaling events in cardiac muscle contraction. It acts as a Ca^{2+}-dependent switch, activating and deactivating the myofilament leading to contraction and relaxation of the muscle cell. An important mechanism in the regulation of contraction is the opening up of the TnC hydrophobic patch to allow TnI to bind. We performed microsecond molecular dynamics simulations of TnC and two of its mutants (V44Q, E40A) in different states of calcium